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Electromagnetic Energy and *Physarum*

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INTRODUCTION

Fourteen years ago we initiated a program of research aimed at understanding the nature of the interaction between weak, low-frequency electromagnetic fields (EMFs) and biological organisms. We selected for study the slime mold *Physarum polycephalum* because it was an extremely well-characterized organism with a well-defined, easy-to-measure cell cycle (1-3). The field intensities and frequencies selected for examination were to a large extent dictated by a need in the early 1970's for developing a basis for assessing the hazards of exposure to weak fields. In recent years we have moved to emphasize mechanisms of interaction between the fields and organisms. This reflects both a change in attitude and in research strategy, since today there is no question that fields can affect organisms.

Our early work was funded by the US Navy as part of their research program related to potential bioeffects of EMFs associated with what was then called Project Sanguine. Project Sanguine was the name given to a large communication antenna that would be buried underground across hundreds of square miles in Northern Wisconsin and which would emit extremely-low-frequency electromagnetic radiation at 45–80 Hz. The project concept has changed design and names and its scope has been reduced several times since 1970, and is now referred to as Project ELF.

The antenna is designed for communication with submerged submarines and takes advantage of the inverse relationship between absorption of EMFs by water and the frequency of the fields. A dual antenna system located in Northern Wisconsin and Upper Michigan can communicate with submarines throughout the world. The field strengths in the ground near the Sanguine antenna as originally designed were expected to be a maximum of 0.07 V/m and 20 μT (the present antenna design does not involve a buried antenna, so lower ground fields than these will occur). Our experiments were designed to examine effects of fields up to ten times stronger than the proposed maximum fields. The initial values of the field for experimentation were thus chosen to be 0.7 V/m and 0.2 mT, rms, measured in the growth medium. The applied sinusoidal fields were in phase, as one would expect near the antenna.

Because of the low intensity of the electromagnetic fields involved, we reasoned that

any observed bioeffect would probably be subtle. This thinking has guided much of our research; its application has resulted in the expenditure of a great deal of time and effort to obtain optimum resolution from every technique applied to the study of EMF bioeffects.

EXPOSURE TO ELECTROMAGNETIC FIELDS

Our initial experiments were designed to examine the effects of EMFs on the timing of the nuclear division cycle. Two incubators equivalent in all respects were used. One incubator served as a control environment and was equipped with an exposure sys tern that was not energized; the second incubator was used to subject *Physarum* to the EMFs being examined. The incubators were chambers could disconnecting a designed so that the control and experimental be randomly switched by simply energizing or disconnecting a few circuits. Routine switching of control and experimental incubators was done to control for subtle incubator differences that might produce artifacts. A master-slave circuit was installed between the growth chambers that minimized temperature differences to less than 0.3°C (4). The fan motors supplied with the incubators were replaced with induction motors to reduce ambient magnetic fields.

An exposure vessel was designed and constructed that would facilitate optimal growth of the organism while providing for easy application of the fields. Electric fields were applied by using stainless steel electrodes placed in direct contact with the medium (Figure 1). Placement of the electrodes in direct contact with the medium assured us of being able to specify the internal fields far more precisely than if the electrodes were separated from the growth medium by an insulator. This design introduced the possibility that electrolysis products could be generated. To test for the possibility that electrolysis products might affect cells grown in these vessels we exposed batches of sterile growth medium to fields of 0.7 V/m and 0.2 mT for 48 hours without cells present. Unexposed cells were then added to this medium for 24 hours and the timing of mitosis was compared with control cultures grown in medium that had been placed in flasks 48 hours earlier but not previously exposed to fields. No differences were detected (5). Magnetic fields were generated by coils placed inside the incubator surrounding a shaker and a platform for Petri dishes (Figure 2).

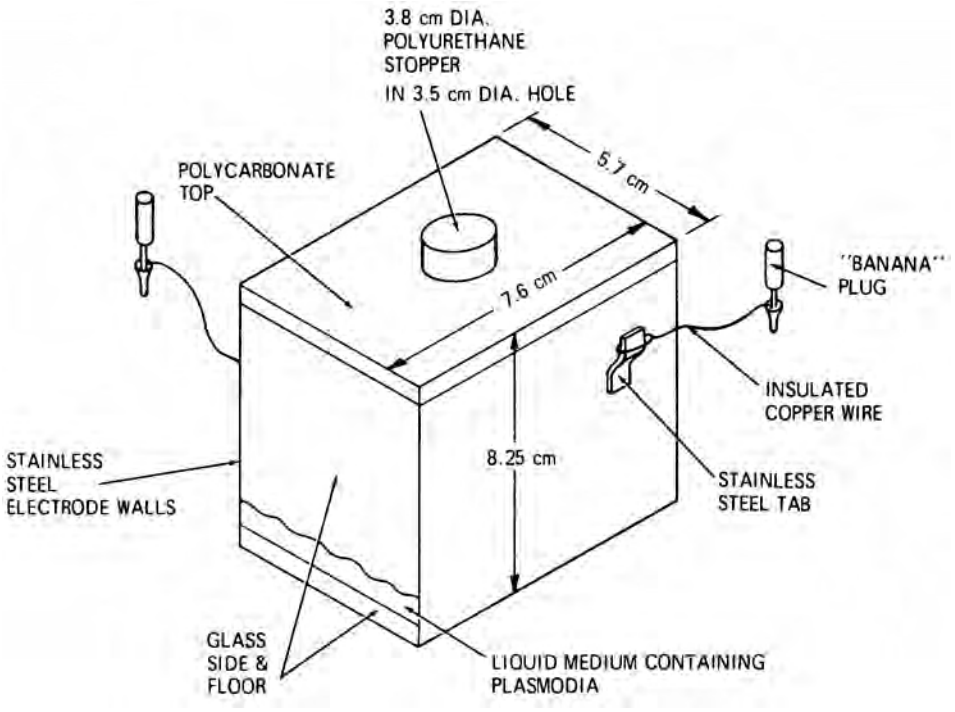

Figure 1. Rectangular flask used to maintain submerged shaker flask microplasmodia and amoebae. Parallel stainless steel electrodes comprise the sides of the vessel. The bottom and ends are made of glass, and the top is constructed of polycarbonate. A foam plug maintains sterility.

NUCLEAR DIVISION CYCLE

Experiments to assess the effects of weak fields were initiated by subculturing non-exposed, submerged, shake flask microplasmodia into eight exposure flasks: four cultures were maintained as controls and the remaining four were placed in the EMF-exposure incubator. Our first set of experiments sought to ascertain the effects of simultaneous 75-Hz electric and magnetic fields of 0.7 V/m and 0.2 mT on the length of the mitotic cycle. Control and EMF-exposed cultures were maintained in the appropriate incubators as submerged microplasmodia, and the length of their mitotic cycles was routinely measured. Cell-cycle measurements were performed using techniques that had been previously developed and used in earlier research (1,2). Aliquots of submerged microplasmodia in log-phase growth were placed on filter paper in Petri dishes and allowed to coalesce for 30 minutes. Following coalescence, nutrient medium was added to each dish, the time noted as the start of the experiment, and the cultures returned to the appropriate incubators. The Petri dish lids incorporated stainless-steel electrodes which were maintained in contact with the filter paper (5). Petri dishes in the EMF incubator were connected to field-generation equipment to continue field exposure over the next 24 hours during which the cell-cycle measurements were conducted; dishes in the control

incubator were connected to unenergized circuits. The coalesced macroplasmodia were discarded upon completion of an experiment.

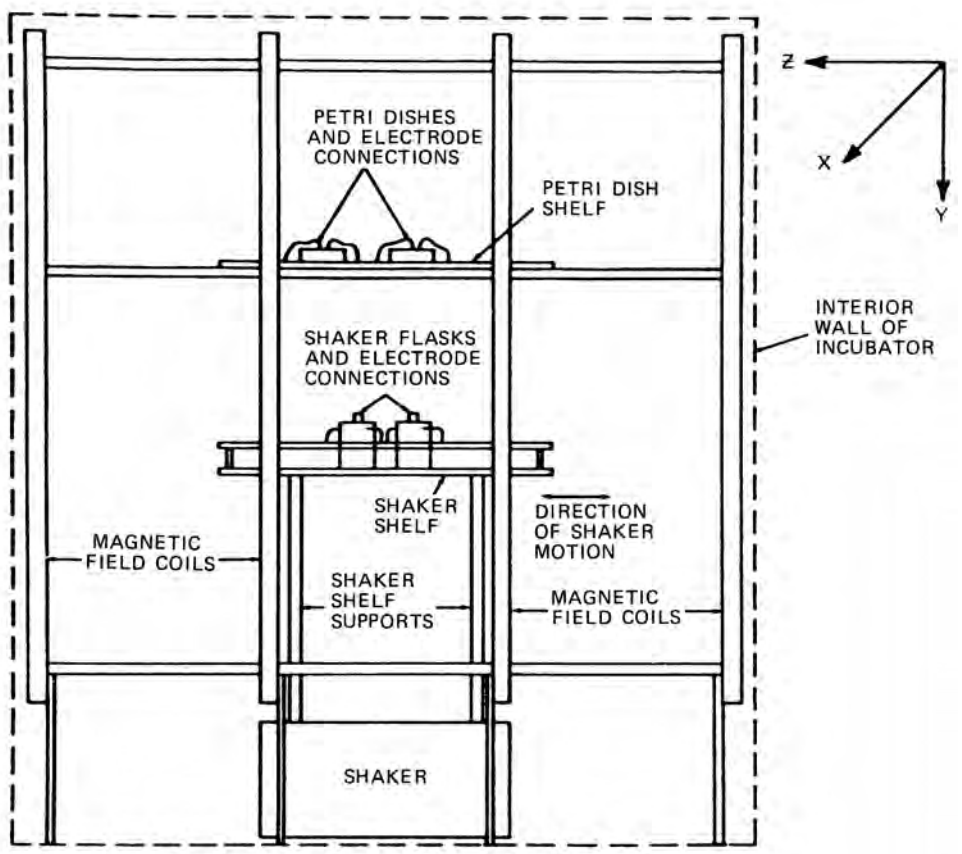

Figure 2. General view of the interior of an incubator. Coils surrounding the cultures generate the magnetic field. The electric fields are individually applied to each culture.

The time required for each culture to reach the metaphase configuration of the second post-fusion mitotic division was determined using ethanol-fixed smears that were scored with phase-contrast optics. In our first experiments we found that after about 120 days of constant EMF exposure, the mitotic cycle was lengthened by 30–60 min relative to control cultures (Figure 3). The exact length of exposure required to induce this effect could not be precisely determined; we used the criterion that a statistically significant difference in the mitotic cycle must occur for three consecutive experiments before we accepted the possibility that an effect had occurred. The experiments were continued for various time periods to statistically establish the occurrence of an EMF effect. After a significant effect was established, a new set of four cultures was introduced into the EMF incubator, and the experiment was repeated. In all experiments, a conclusion that an EMF effect had occurred was accepted only if the alteration could be reproduced with a new set of cultures at least once (5).

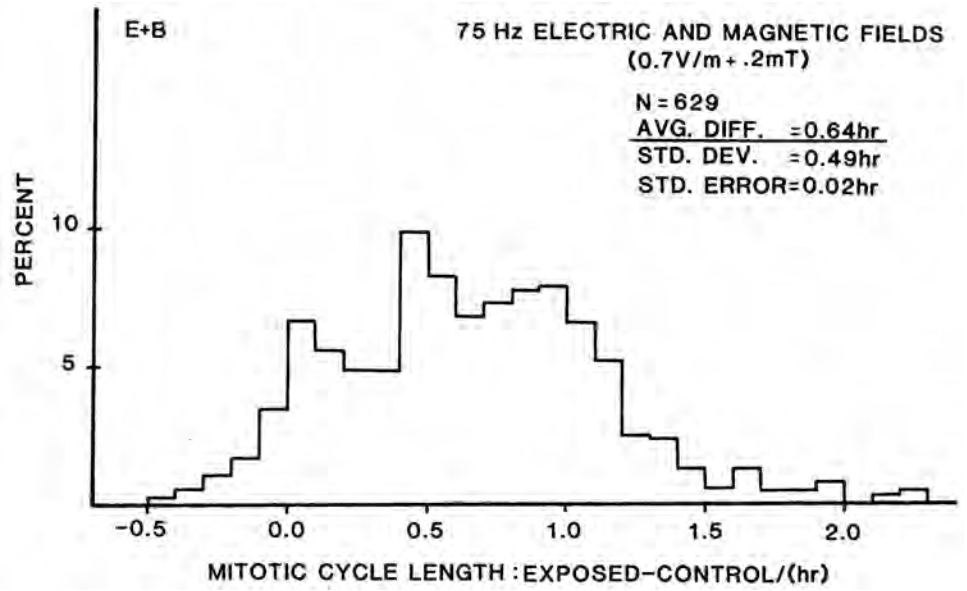

Figure 3. Distribution of differences in the length of the mitotic cycle for exposed cultures relative to the average daily control cell cycle (7). The average control value for each day has been subtracted from each observation made on exposed cultures. N is the total number of observations on exposed cultures represented in the histogram. Field-exposure conditions and histogram statistics are shown.

After we had repeated the observation of a longer cell cycle in cells exposed to 75-Hz fields, we changed the applied frequency first to 45 Hz and later to 60 Hz, while maintaining field intensities at 0.7 V/m and 0.2 mT. In these experiments the mitotic cycle was lengthened to a degree almost identical to that observed in cells exposed to 75-Hz fields. The only apparent difference appeared to be that the lower frequency fields required slightly less time to produce an effect. Unfortunately, this observation could not be more accurately quantified because we lack an unambiguous method for assessing the initial responses of *Physarum* to EMF exposure.

One of our more interesting cell-cycle experiments involved a series of cell fusions (6). In these experiments microplasmodia from control and EMF-exposed cultures were mixed in varying proportions and allowed to coalesce to form a mixed-nuclear macroplasmodium before timing their cell cycle (Table 1). Even though cells with different cell-cycle lengths were mixed to form a single syncytium, all nuclei within the syncytium underwent mitosis synchronously in the manner characteristic of *Physarum*. When control and EMF-exposed microplasmodia were mixed in a 1:1 ratio, mitosis of the mixed culture occurred somewhat earlier than the time predicted by computing a simple average of the cycle lengths of the two unmixed cultures. Mixing control and experimental cultures in a ratio of 3 to 1 respectively resulted in a cell-cycle length that

Table 1. Results of Mixing Experiments. The standard error of the mean is given in parentheses.

Weight Ratio (Control/ Exposed)	No. Days Data	Total Number Cultures	Average Daily Fractional Shift*	Average Time to Second Metaphase (hr)		
				Control	Mixture	Exposed
1:3	4	60	69 (22%)	14.99 (.09)	15.38 (.12)	15.60 (.08)
1:2	22	320	48 (6%)	14.62 (.03)	14.99 (.04)	15.47 (.04)
1:1.5	4	57	40 (14%)	15.21 (.10)	15.53 (.08)	16.04 (.05)
1:1	6	89	13 (9%)	15.05 (.11)	15.15 (.12)	15.69 (.14)
3:1	4	60	3 (14%)	14.65 (.10)	14.68 (.12)	15.28 (.09)

*$[(M–C)/(E–C)] \times 100$

did not differ significantly from that of the control. In contrast, when the ratio of the mix was reversed (1 part control, 3 parts EMF-exposed), the length of the cell-cycle was significantly shorter compared to exposed plasmodia. We concluded from these and other mixing experiments that the control contribution to the combined plasmodium exerts a stronger influence on the mixtures than does the contribution from exposed plasmodia. One possible explanation is that control cultures have an excess of some factor(s) involved in mitosis that is in lower concentration in the experimental cultures. Whatever the reason, these experiments provide unequivocal evidence that cultures exposed to these weak electromagnetic fields exhibit significantly changed cell-cycle characteristics.

INDIVIDUAL ELECTRIC AND MAGNETIC FIELDS

Having convinced ourselves that lengthening of cell cycle was not an artifact, we proceeded to examine the role of the individual electric (E) and magnetic (B) fields, and to determine whether both were required to produce an effect (7). To address this question, cultures were exposed to either a 75-Hz E-field of 0.7 V/m, or a 75-Hz B-field of 0.2 mT, or to combined fields of the same strengths. The E+B experiment was a replication of the earlier work. When both fields were simultaneously applied the exposed cultures had a cell cycle that was lengthened by 0.64 hr. The cell cycle in cultures exposed only to B was lengthened by 0.46 hr; cultures exposed only to E were also lengthened by 0.39 hr (Figures 4 and 5). The smaller delay for either single-field exposure is significantly different from the combined-field exposures.

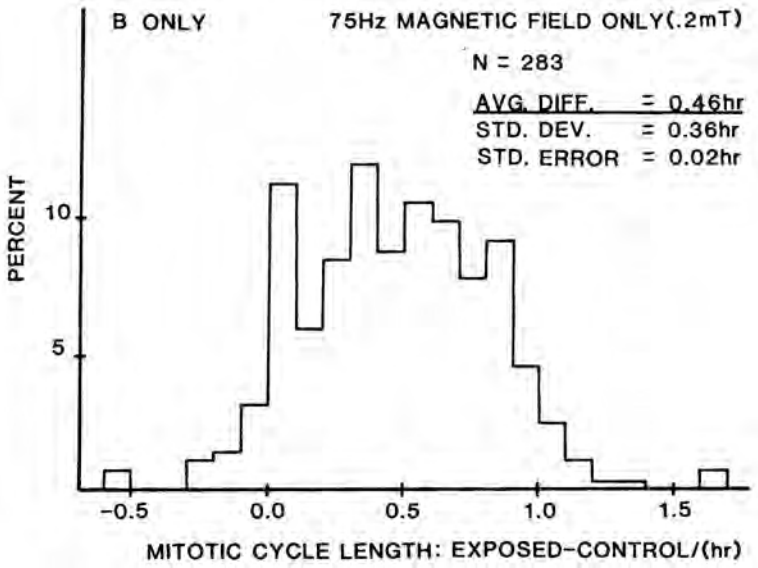

Figure 4. Distribution of differences in the length of the mitotic cycle for cultures exposed to 0.2 mT (7).

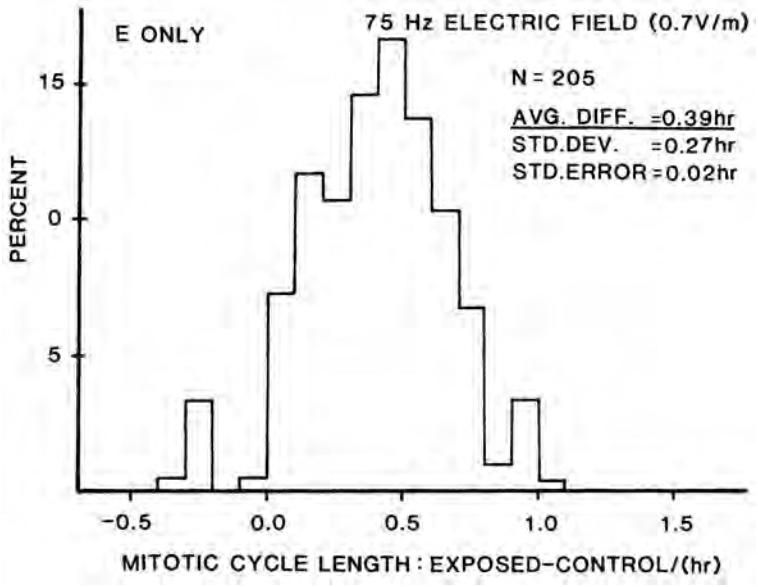

Figure 5. Distribution of differences in the length of the mitotic cycle for cultures exposed to 0.7 V/m (7).

These data demonstrate individual E and B fields can slow the mitotic cycle, but that the effects of the individual fields do not appear to be additive. We regard the observation that electric and magnetic fields can each alter the cell cycle in nearly the same way as one of our most important observations. It presents a severe test to any mechanism proposed to account for these findings. There may well be two separate mechanisms that work either in concert or individually, to produce a lengthening of the cell cycle. Our

work with pulsed electric and magnetic fields discussed below supports these observations.

NO LOWER THRESHOLD OBSERVED

We attempted to determine if a lower threshold existed by decreasing the field intensities by a factor of five to 0.14 V/m and 40 μT. The data (Figure 6) showed that the mitotic cycle was lengthened by 0.42 hr. Thus application of fields five-fold weaker induced a significantly smaller delay that is about the same magnitude as a more intense E field alone (0.39 hr) or an B field alone (0.46 hr).

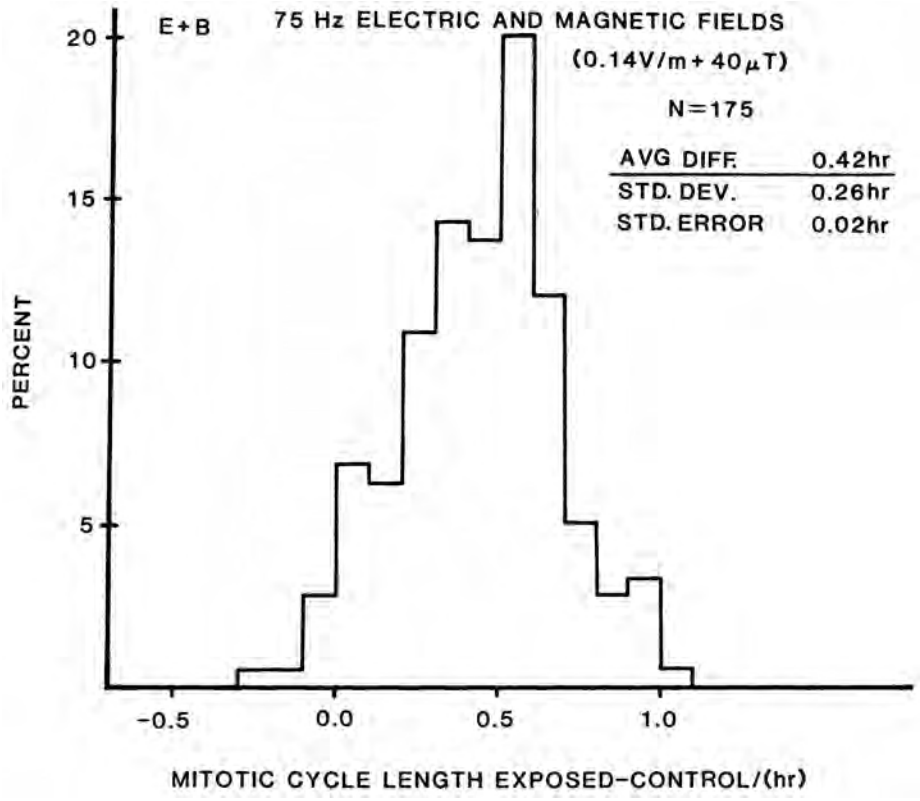

Figure 6. Distribution of differences in the length of the mitotic cycle for cultures exposed to 0.14 V/m and 40 μT (7).

In a related experiment using a modulated waveform quite similar to the sinusoidal 75-Hz fields, the applied field intensities were decreased by an additional factor of four to 0.035 V/m and 10 μT; a delay of 0.43 hr was obtained (Figure 9). Because we only performed experiments applying both the E and B fields, we are unable to determine whether a threshold for either the E field or B field was reached. Based on these data a dose-response relationship was not observed. In particular, a 20-fold decrease in the applied field intensities did not result in a proportional shortening of the mitotic delay.

MODULATED WAVEFORM

We also performed several experiments in which the wave form was modulated by shifting the frequency at the waveform peaks so that no discontinuities were introduced into the wave (7). This type of frequency modulation is known as minimum-shift-keying modulation and was selected because it had been proposed for use with the Sanguine/ ELF communication system. In these experiments, the oscillator driving the field-generation equipment was set at a nominal center frequency of 76 Hz. It made random frequency shifts between 72 and 80 Hz on the average of eight times a second. Three pairs of field intensities were examined: (a) 0.7 V/m and 0.2 mT, (b) 0.14 V/m and 40 μT, and (c) 0.035 V/m and 10 μT. The results are shown in Figures 7–9. We conclude from these data that this type of modulation adds nothing new to the fixed-frequency sinusoidal fields used in earlier experiments.

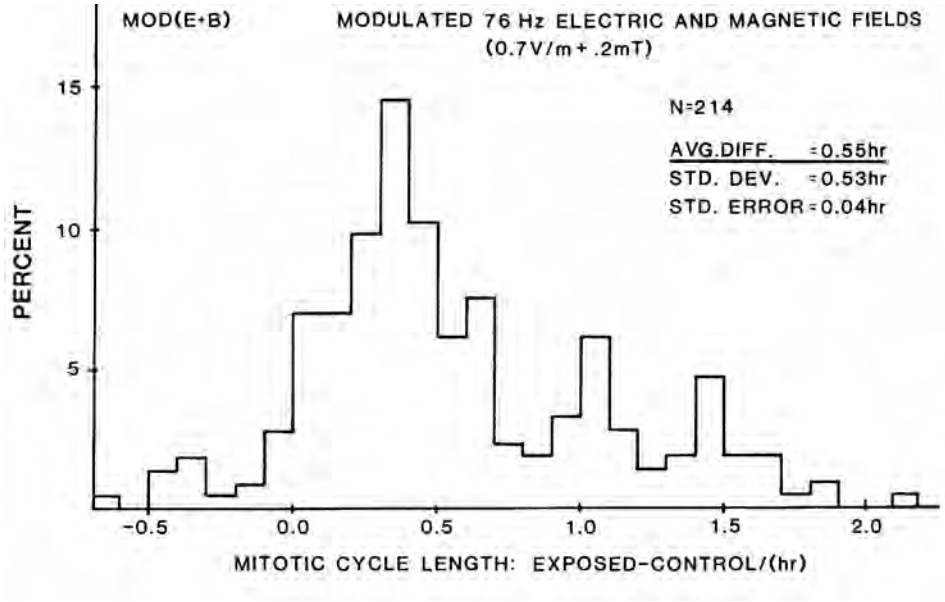

Figure 7. Distribution of differences in the length of the mitotic cycle for cultures exposed to modulated fields of 0.7 V/m and 0.2 mT (7).

CONSISTENCY AND REPRODUCIBILITY

To insure internal consistency and reproducibility of the results, all data sets were analyzed by both a paired t test and a Wilcoxon signed-rank test (8). Both tests were used to compare two distributions of data to see if the means differed, or if the mean of a distribution differed from zero. The results of these comparisons are presented in Figure 10 (7). Reproducibility was tested by constructing distributions for each individual set of cultures and comparing them with one another. Internal consistency of the data was examined by comparing a culture set with its replicate or by comparing the results of

equivalent tests such as Bl vs El and B2 vs El. The results of all such combinations are shown in Figure 11 (7). Based on these analyses, we conclude that the data are both reproducible and internally consistent.

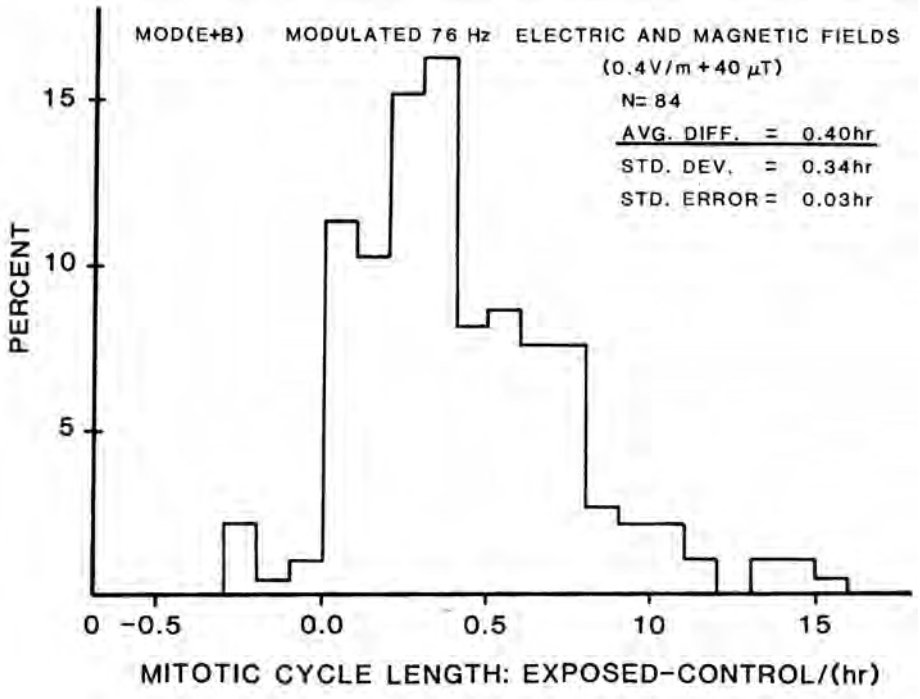

Figure 8. Distribution of differences in the length of the mitotic cycle for cultures exposed to modulated fields of 0.14 V/m and 40 μT (7).

RESPIRATION

EMF effects on respiration were also examined. In these experiments the same EMF-exposed and control microplasmodia that were used for the mitosis experiments were used to determine the respiratory quotient QO_2 (in units of microliters of O2 consumed/min/mg protein). In our initial experiments we used a Warburg apparatus to measure respiration (7); in later experiments a Clark-type oxygen electrode was employed. Microplasmodia exposed to 75-Hz fields of 0.7 V/m and 0.2 mT exhibited a 16% decrease in QO_2 relative to controls. Exposure to either E or B fields alone decreased respiration by 8% and 9%, respectively. Application of modulated 76-Hz fields of 0.7 V/m and 0.2 mT produced a 7% decrease in QO_2, while weaker modulated fields of 0.14 V/m and 40 μT or .035 V/m and 10 μT diminished QO_2 by 3% and 4%, respectively (Figure 12).

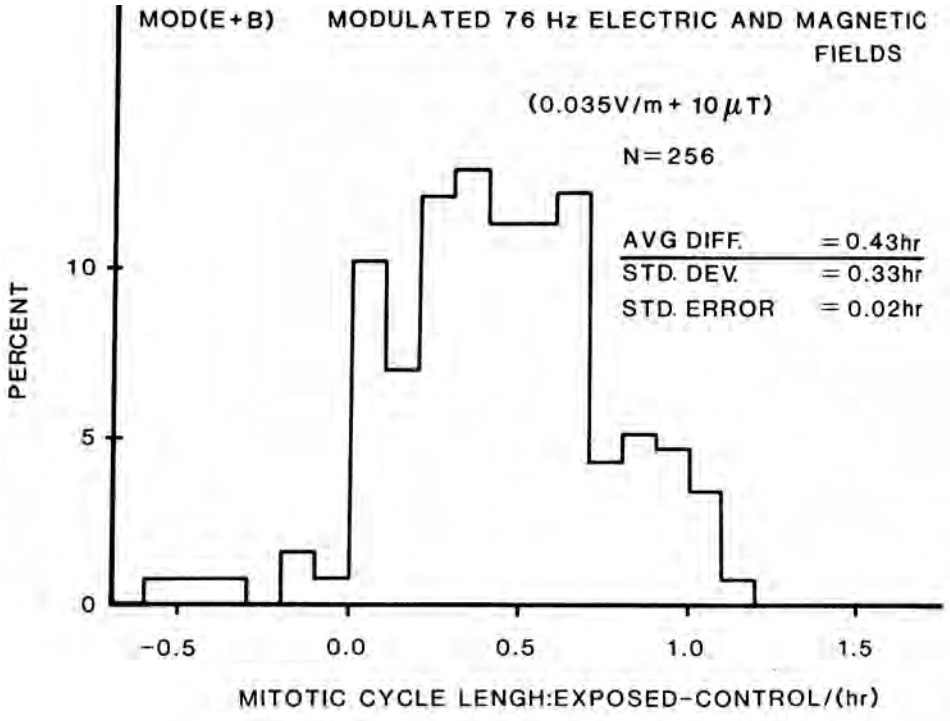

Figure 9. Distribution of differences in the length of the mitotic cycle for cultures exposed to modulated fields of 0.035 V/m and 10 μT (7).

These data show that EMF exposure resulted in a physiological depression in the respiration rate of the cell and, in general, they are consistent with our earlier cell-cycle experiments. However, whereas the individually-applied fixed-frequency E and B fields were not additive in their effects on the cell cycle, they appear to be additive in their effects on respiration. It is noteworthy that the magnitude of the depression was less in cells exposed to fields of modulated frequencies than in those exposed to a fixed-frequency field. These conclusions are weak, and may be biased by the broad distribution seen in the data obtained using modulated fields.

EFFECTS WITH TIME

Once an EMF effect has been induced in microplasmodia, its magnitude neither increases nor decreases with time. This fact has been substantiated by observing cultures that have been continuously exposed to EMF for periods as long as 5 years. To assess the ability of affected cultures to return to baseline levels, cultures exhibiting a lengthened mitotic cycle were removed from the field and placed in a control environment. The persistence of the lengthened mitotic cycle was periodically assessed; the results are shown in Figure 13. Within about 30 days, the mitotic cycle slowly shortened to the length of the control cycle. If the relaxed cultures were returned to the EMF environment, a lengthened mitotic cycle was re-established in about 30 days instead of about the 120 day period required when the cultures were initially exposed to EMFs.

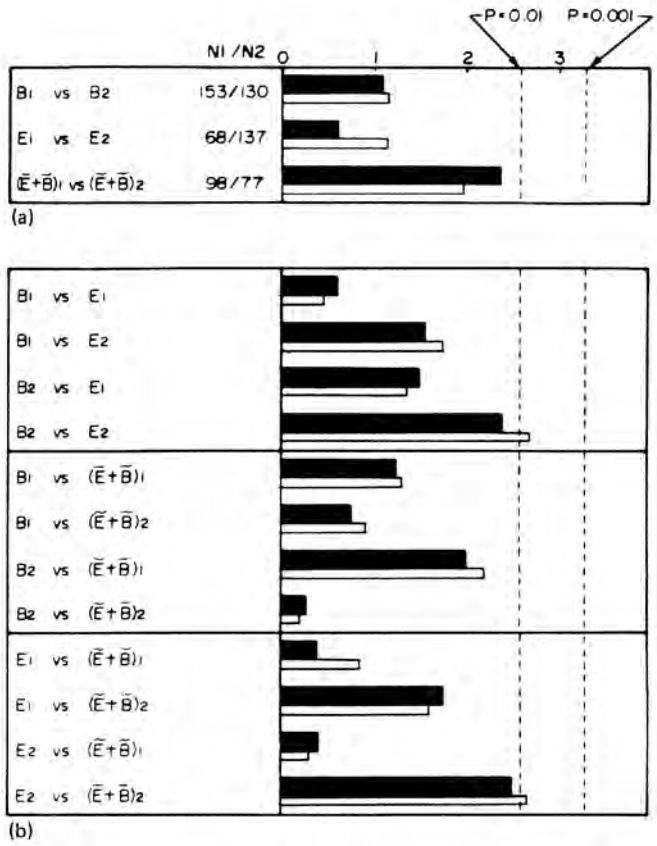

Figure 10. Statistical comparison of distributions to test reproducibility and internal consistency of the data. The solid bar gives the results for the Wilcoxon signed ranks test; the open bar gives the t-test statistic. The P = 0.01 and P = 0.001 levels for large samples are shown. (a) Test for data reproducibility by comparing distributions of cycle length data for two sets of cultures each exposed to the same field conditions but at different times. For example, cultures in set B_1 were exposed to a 75-Hz magnetic field of 0.1 mT; cultures in set B_2 were also exposed to a 75-Hz magnetic field of 0.1 mT but these experiments began almost 1 year later. N1/N2 is the number of data in the first/second set. (b) Test of internal consistency by comparison of equivalent distributions for different field conditions. There are three sets of four comparisons, all of which display internal consistency. Note also that the distributions are all statistically equivalent (7). Here, and in Figures 11 and 12, E = 0.7 V/m, B = 0.2 mT, = 0.14 V/m, = 40 μT, = 0.035 V/m, = 10 μT. The subscripts refer to replicate experiments.

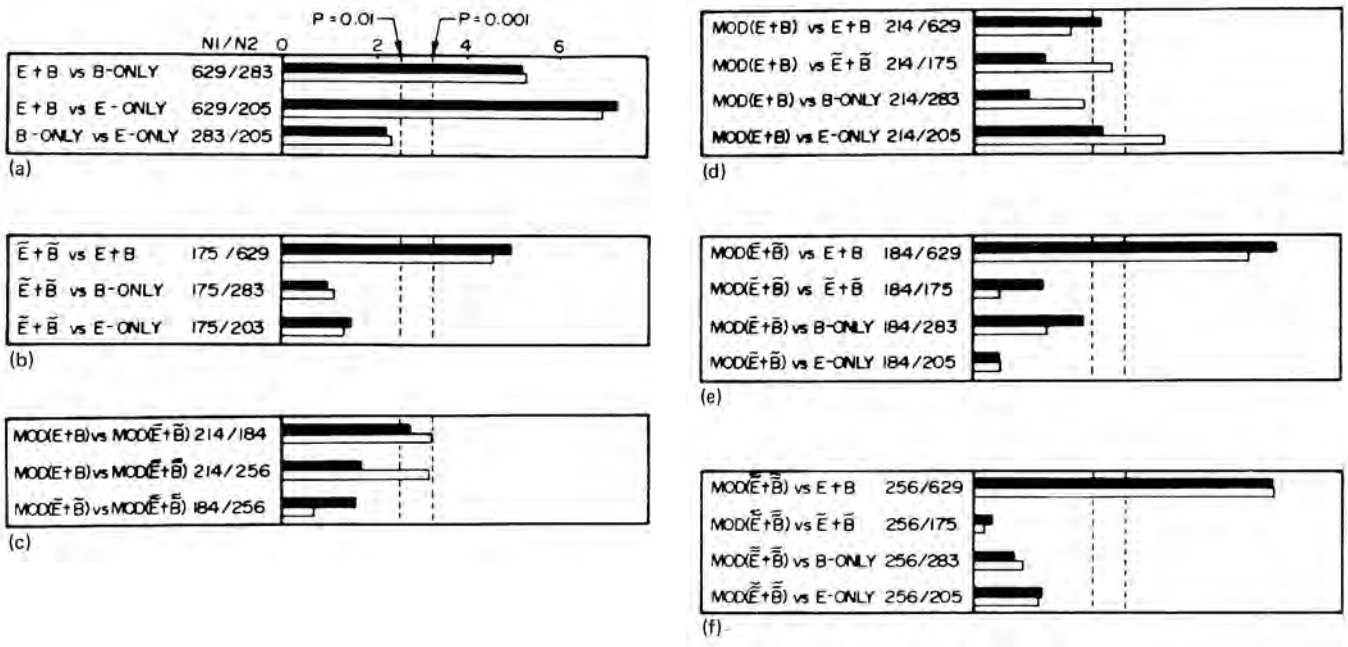

Figure 11. Comparison of mitotic cycle-length distributions for different field conditions. Data are pooled so that all data taken at a particular set of field conditions are treated as a single group. The solid bar gives the results for the Wilcoxon signed-rank test; the open bar gives the t-test statistic. The P = 0.01 and P = 0.001 levels for large samples are shown on the graph as vertical lines. Entries in the column headed by N1/N1 are the number of data in the first/second distribution. (a) Conclusions: E + B ≠ E-only or B-only. Simultaneous application of electric and magnetic fields produces a different mitotic cycle lengthening distribution than that observed when fields are applied individually. At these levels (0.2 mT and 0.7 V/m) the individual fields produce statistically identical results. (b) Conclusions: + = E-only and B-only; + ≠ E + B. Electric and magnetic fields applied at a level five times weaker cause a different result to occur. These weaker fields produce the same effect as either more intense E fields alone or more intense B fields alone. This finding agrees with those of Figure 10. (c) Conclusions: MOD(E + B) ≠ MOD(); MOD(E + B) = MOD(); MOD() = MOD(). Reduction in field intensity by five times causes the mitotic cycle lengthening distribution to change, a further decrease in intensity by four times has no effect. (d) Conclusions: MOD(E + B) = E + B = = B-only ≠ E-only. Modulation of the fields results in a mitotic cycle lengthening distribution similar to unmodulated fields of weaker intensity. Unlike the unmodulated fields, the MOD(E + B) results are similar to the B-only exposures. (e) Conclusions: MOD() ≠ E + B; MOD() = ; MOD() = B-only or E-only. Modulated fields at 0.04 V/m and 40 μT produce results statistically identical to unmodulated fields of the same intensity. Like the unmodulated fields, MOD() data are indistinguishable from those produced by more intense unmodulated E-only and B-only fields. (f) Conclusions: MOD() ≠ E + B; MOD() = ; MOD() = B-only or E-only. Decreasing field intensities further to 0.035 V/m and 10 μT has no apparent effect on the mitotic cycle lengthening distribution (7). MOD, modulated by minimum-shift-keying.

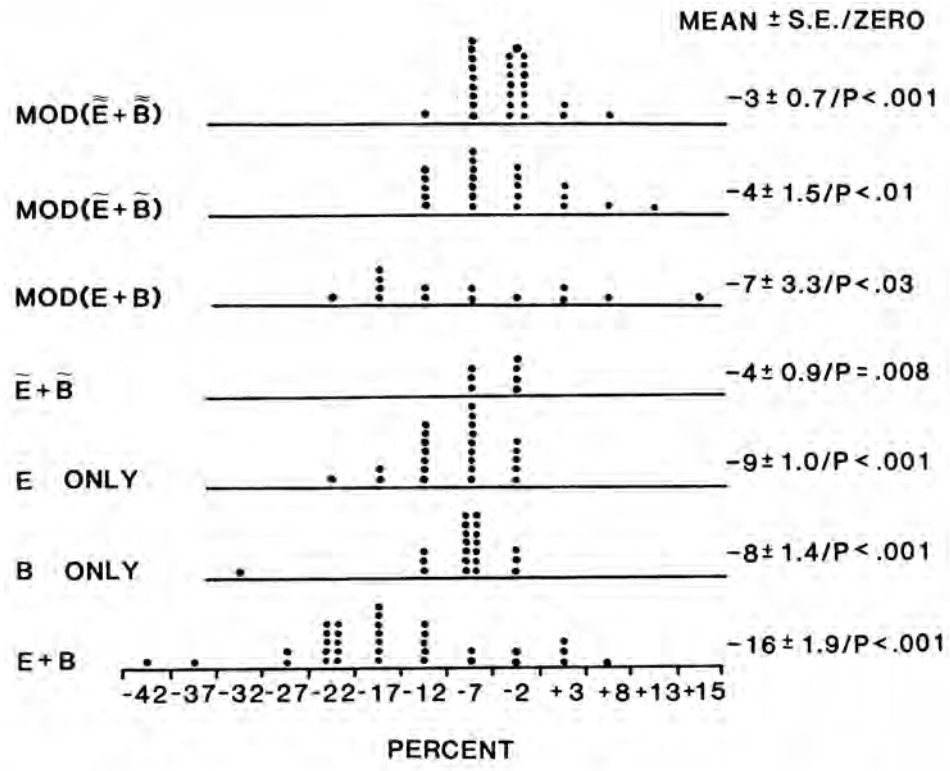

Figure 12. Respiration rates for exposed microplasmodial cultures relative to average daily control-culture respiration rates. Histogram bins are 5% wide; the center value of the bi n is given below on the abscissa. Each point represents a set of rate measurements performed upon a single, exposed culture taken relative to the average of rate measurements in two control cultures determined at the same time. Data given on the right are average values ± standard errors computed from the raw data. The probability that the distribution mean differs from zero only by chance is also listed; these probabilities are derived using the Wilcoxon signed-rank statistic.

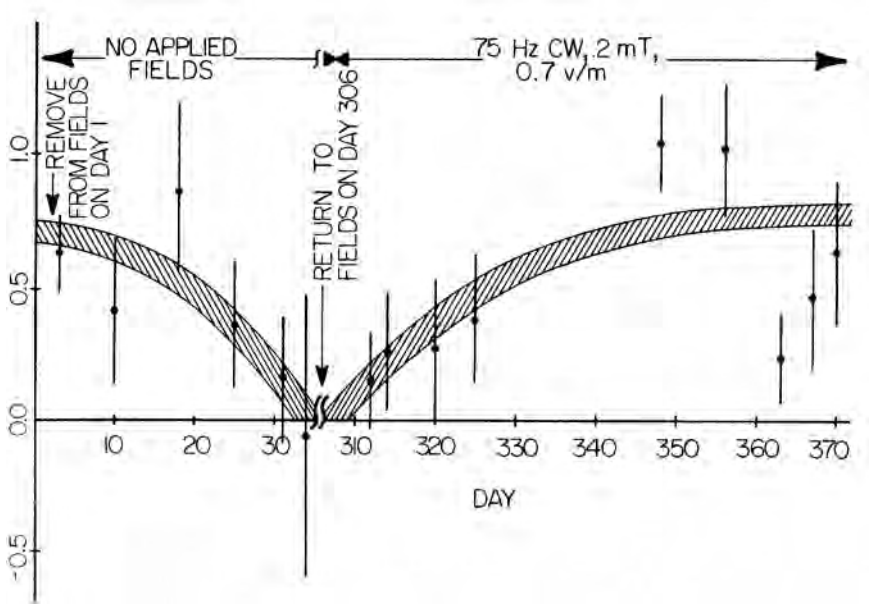

FIGURE 13. Microplasmodia exposed to 75 Hz, 0.2 mT, 0.7 V/m for 270 days and showing a lengthening of the mitotic cycle relative to control cultures. Ordinate shows the average difference of the nuclear division cycle from the time nutrient medium is added to a fused microplasmodium to the second metaphase configuration of the nuclei. Each point represents the average of 10 EMF-exposed cultures from which the average of 10 controls has been subtracted. Placing the EMF-exposed cultures into a control environment on day 1 (day 270 of exposure) resulted in the slow dissipation of the mitotic delay over a 30-day period. If the cells were reintroduced into the field (day 306), a mitotic delay became significant within 30 days.

CHROMOSOME EFFECTS

We also examined whether chronic exposure could induce a measurable drift in chromosome number. Since *Physarum* is a polyploid organism, distribution of chromosomes must be measured. Although the small size of the chromosome makes it extremely difficult to perform direct measurements on chromosome spreads, a distribution in the nuclear sizes may be examined for any gross change in nuclear material. The methods employed were based on the work of Mohberg et al. (9), who established that the distribution of nuclear sizes in *Physarum* is closely related to the distribution of chromosome numbers. Isolated nuclei were photographed under a phase-contrast microscope and the distribution of nuclear diameters were measured.

Measurements of exposed nuclei (75 Hz, 0,7 V/m, 0.2 mT, N = 104) yielded a mean diameter of 3.16 ± 0.05 μm (±95% confidence limits); the mean diameter of control nuclei (N = 213) was 3.14 ± 0.03 μm. These two means are not significantly different from each other. An estimate of how large the difference between the means would have to be before it could be detected can be gotten using the power function of the t test (8).

This function estimates the sensitivity of a test from the scatter in the data at a predetermined confidence level. Variability in our data for nuclear diameters is such that we should be able to detect a difference as small as 0.09 μm between the means with 95% confidence; this difference corresponds to 3% of the mean values.

An interesting figure for comparison is the maximum difference in chromosome number. Using the measurements of Mohberg et al. (9) we found an empirical relationship between diameter, d, in micrometers, and chromosome number, c. Their data were fitted to first-, second-, and third-order equations, and the best fit was found for the linear equation $c = 41.2(d - 1.78)$. The fit for different powers of the mean diameter was nearly the same as measured by the correlation coefficient; 0.983 (1st power), 0.980 (2nd power), and 0.971 (3rd power). Based on these data we conclude that if a difference in chromosome number existed between exposed and control nuclei, it was less than 4 out of a total of approximately 60 chromosomes ($P < 0.05$).

The result of the chromosome measurements does not, however, rule out subtle changes at the level of the genes. An argument against the observed effects being attributable to slow, spontaneous genetic drift can be made based on the data concerning reproducibility of our mitotic cycle and respiration experiments described above. We have repeatedly placed sets of new stock cultures derived from our main stock control cultures into EMF environments. In all cases the effects induced were consistent with those induced in cultures introduced at other times. Any genetic drift affecting the measured parameters that does not occur reproducibly under the influence of EMF should create discernible differences among the various sets of cultures exposed to the same EMF environments.

SEARCH FOR A MECHANISM

As these data accumulated, we began to ponder the type of mechanism that might explain how such weak fields could interact with cells. A magnetic field of 0.2 mT produces a maximum current density of less than 10^{-5} A/m^2 in the exposure flasks, while an electric field of 0.7 V/m produces a current density of 0.55 A/m^2. Regardless of the mechanism invoked, it is difficult to imagine a scenario in which such weak fields or low current densities could produce a significant impact on a biological system. From a purely theoretical standpoint, it is improbable that both field components are interacting with a cell via the same mechanism. Based on a consideration of the current densities of the applied electric field and the impedance of the membrane, one may conclude that little or no current crosses the plasma membrane. In contrast:, the membrane presents no barrier to the applied magnetic field. Since the individually applied fields can each induce a bioeffect, it seems probable that more than one mechanism of interaction is involved in producing the EMF effects we have observed.

One of our first experimental attempts at elucidating the mechanism of EMF interaction was aimed at determining whether DNA replication in the diploid plasmodium of *Physarum* was altered. Our failure to detect significant differences in DNA levels in exposed cells set the tone for numerous future experiments. Whenever we attempted to examine a specific biochemical phenomenon we were unable to demonstrate an EMF response. In our laboratory, using our experimental system, EMF effects are more easily demonstrated in plasmodia if we examine parameters that represent the sum of numerous physiological processes. But a word of caution needs to be added to this observation by restating the obvious. Failure to demonstrate a significant effect may also be attributed to the resolution of the technique and cannot be construed as evidence that the parameter in question is not affected by EMFs.

AMOEBAL CELL PHYSIOLOGY

We realized the necessity of attempting to understand the mechanism of interaction between weak fields and cells, and chose to concentrate on the most likely initial site, the cell membrane. Because the plasmodium has a thick layer of slime (glycocalyx) surrounding its plasma membrane, we decided that the haploid amoeba stage of *Physarum* which lacks a readily discernible glycocalyx would be a more suitable model system for these studies. Amoebae (RSD–4) were grown in axenic medium in suspension culture and exposed to EMFs using the same exposure system described above. *Physarum* amoebae are 10–15μm in diameter, have a doubling time in suspension culture of about 27 hours and, unlike the plasmodium, they undergo both karyokinesis and cytokinesis. Thus, in many respects they are more representative of a mammalian cell than is the plasmodium.

In our initial studies with amoebae, they were exposed to 1.0 V/m and 0.1 mT at 60 Hz (10). Subsequent experiments involved exposure to the individual fields. The results of QO_2 measurements made using an oxygen electrode are shown in Table 2. Respiration was depressed 20% in cells exposed to E+B fields, 7% in cells exposed to E fields alone,

Table 2. QO_2 (µl O_2 consumed/cell/min) in Amoebae Exposed to 60-Hz Sinusoidal Electromagnetic Fields

	E & B		**E Only**		**B Only**	
	Control	**1.0 V/m, 0.1 mT**	**Control**	**1.0 V/m, 0.1 mT**	**Control**	**1.0 V/m, 0.1 mT**
Overall Average	.620	.496	.645	.595	.660	.666
Average Difference	.124		.049		.006	
P (paired t-test)	<0.001		0.003		0.765	
P (randomization)	0.001		0.016		0.768	

Table 3. Concentration of ATP in Amoebae Exposed to 60-Hz Sinusoidal Electromagnetic Fields

	E & B (fmole ATP/cell)		**E Only (fmole ATP/cell)**		**B Only (fmole ATP/cell)**	
	Control	**1.0 V/m, 0.1 mT**	**Control**	**1.0 V/m, 0.1 mT**	**Control**	**1.0 V/m, 0.1 mT**
Overall Average	.496	.459	.509	.452	.492	.459
Average Difference	.037		.057		.033	
P (paired t-test)	<0.007		<0.004		<0.006	
P (randomization)	<0.007		0.002		0.016	

and no significant effects were observed in cells exposed to B fields only. Since mitosis cannot be readily followed in amoebae, we chose to examine the ATP content of exposed cells as an additional probe of the cells' physiology. ATP levels for amoebae exposed to identical field conditions are given in Table 3; 60-Hz E+B fields depressed the cells' ATP content by 8%, whereas an E field alone depressed ATP levels by 11%. A somewhat surprising finding, given our earlier observation of no change in QO_2, is the fact that the B field alone resulted in a 7% decrease in ATP. It should be noted once again that the absence of a significant depression in the QO_2 when a B field alone is applied, means that if an effect exists, it is below the resolution of our technique.

AMOEBAL CELL MEMBRANE

Once we convinced ourselves that amoebae could be affected by exposure to weak EMFs, we decided to examine their cell surface using the technique of cell partitioning in a two-phase aqueous polymer system. Dextran [poly(1,6-glucose)] and PEG [poly(ethylene glycol)] are water-soluble polymers that form a three-component solution of two immiscible phases when placed together at concentrations above a critical point. The upper phase is rich in PEG and the lower phase is Dextran-rich. After cells are introduced into the PEG-Dextran system, the test tube is shaken and allowed to stand; after a short period the cells partition themselves between the interface and the upper phase based on their surface properties.

A partition coefficient is obtained by determining the fraction of the total number of cells located in the top phase at a fixed interval after mixing. Cell partitioning is dependent on the relative affinity of the membrane surface for the PEG-rich solution. The main surface components involved in a cell's affinity for the upper phase are the surface charge and lipid composition.

Data from these experiments showed that exposing amoebae to 60-Hz fields of 1.0 V/m and 0.1 mT resulted in a partition difference of about 7% (Figure 14) (11). These data permit us to conclude that either the amoebae cell surface charge or the cell surface composition or both, have been altered by EMF exposure.

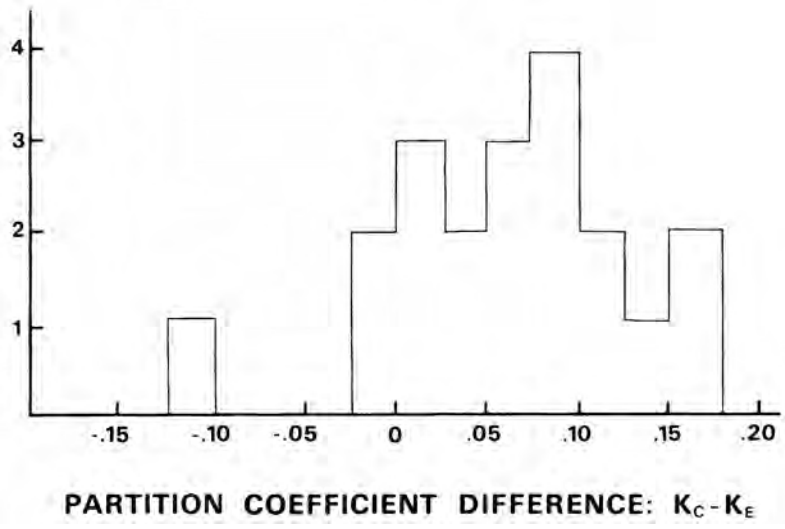

Figure 14. Distribution of differences in partition coefficients for amoebae exposed to EM fields (K_e and control amoebae K_c) note that partition coefficients must assume a value between 0 and 1. The histogram was constructed by computing a difference for each of 20 samples as the average value for the control sample less the average for an exposed sample; the ordinate indicates the number of observations. K_c has an overall average value 0.07 larger than K_e (11).

Having shown that the cell surface is altered as a result of EMF exposure, we began

to examine the partition technique as a probe for approaching and understanding the mechanism of action between EMFs and cells. To amplify the partition differences detected in the single-tube experiments, we applied a technique known as thin-layer countercurrent distribution (TLCCD), the principle of which is shown in Figure 15. Separation is achieved by a stepwise movement of the top phase while the interface and bottom phase remain stationary. In essence, it is a type of cell chromatography where the bottom phase is the stationary phase, and the to p phase is the mobile phase. The partition steps are performed automatically in a 60-chamber circular plate (Bioshef, Sheffield, UK).

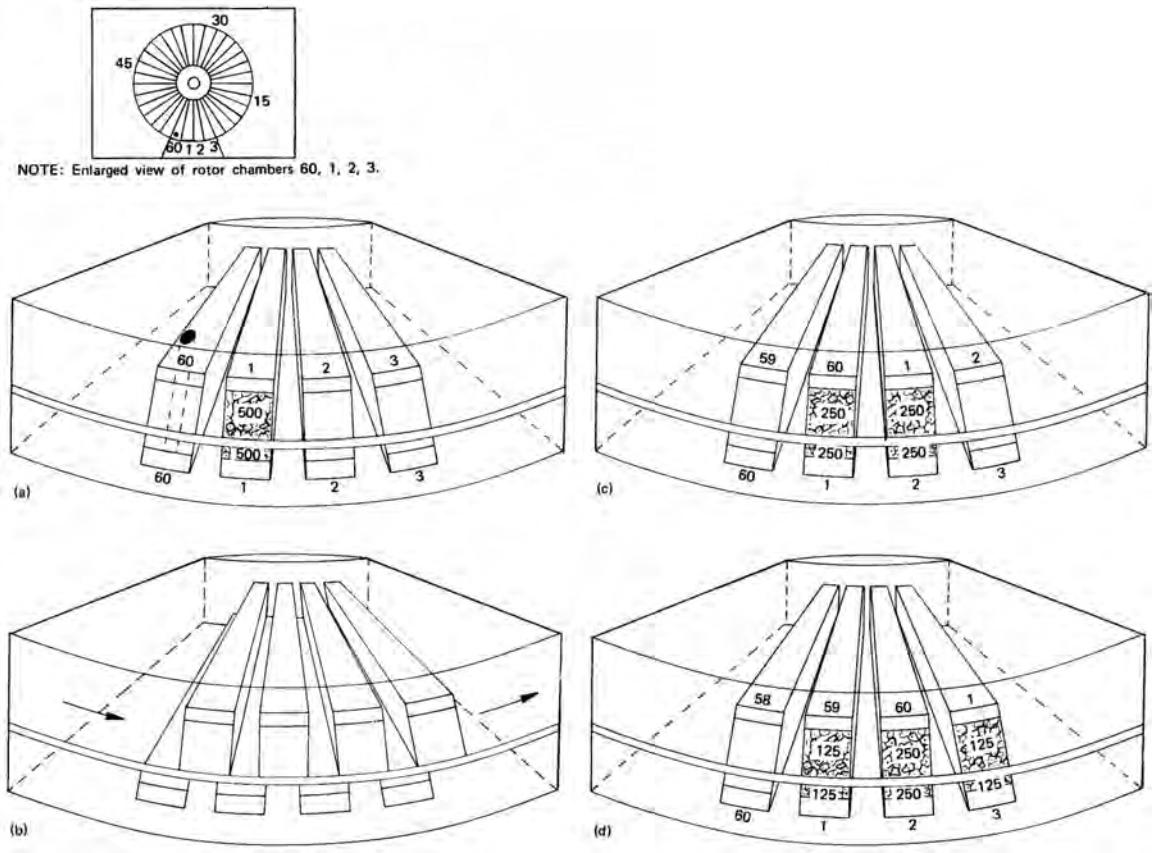

Figure 15. Schematic representation of three steps of a thin-layer countercurrent distribution procedure. Inset at the top is an overview of a 60 chamber rotor. (a) An expanded view of four thin-layer chambers at the beginning of an experiment; chamber 1 contains the original cell load, chambers 60, 2, and 3 contain only upper and lower phase solutions. (b) Following 30 sec shaking period and a 10 min separation period, the top rotor is rotated in a counterclockwise direction by one chamber producing the situation depicted in (c): the 500 cells in the upper phase have been moved to the second chamber and new top phase has been brought into chamber 1. The rotor is shaken again and the phases are allowed to repartition before the top plate is moved counterclockwise by one chamber resulting in the redistribution of cells shown in (d).

Two different phase systems were employed; a high-potential system similar to the one used in the single-tube partitions (5.5% Dextran T-500, 5.5% PE G-8000, 0.05 M/kg phosphate buffer, pH 7.0) and a low-potential system (5.0% Dextran, 4.0% PEG, 0.05 M/kg NaCl, and 0.01 M/kg potassium phosphate). The high-potential system creates a 1–3 mV electrostatic potential difference between the upper and lower phases such that the upper phase is positive relative to the lower phase. The low-potential system has little measurable potential difference between the upper and lower phases so that the cells partition principally on non-charge associated surface properties. Amoebae exposed to 60-Hz fields of 1.0 V/m and 0.1 mT and analyzed simultaneously in both high-potential and low-potential phase systems produced the TLCCD profiles shown in Figures 16a and b. Examination of the pro files shows that the EMF-exposed cells shifted to the right in the high-potential system when compared to control cells, and to the left in the low-potential system. A shift to the right reflects greater attraction for the upper phase.

To examine the effect of the individual electric and magnetic fields, cells were placed in 60-Hz fields of either 1.0 V/m or 100 μT for 24 hours. When the exposed amoebae were analyzed by TLCCD, cells exposed only to an E field were displaced to the right of the controls in the high-potential system but were coincident with the controls in the low-potential system (Figures 16c and d). To our surprise, when amoebae were exposed only to a B field the situation was reversed in two ways: first, the TLCCD profile of magnetic-field-exposed amoebae was coincident with that of the controls in the high-potential system and was displaced to the left in the low-potential system (Figures 16e and f). Thus, an increase in the net surface charge was observed in amoebae exposed to an E field, whereas cells exposed to a B field exhibited a change in non-charge associated surface properties. That is, they showed a decrease in cell surface hydrophobicity. Further, these data show that the individual E and B fields induced different cell-surface alterations.

PULSED EMFs

Pulsed EMFs are widely used in the medical community to facilitate bone repair in clinical cases of non-union. Although this therapeutic regimen appears to be reasonably effective, the mechanism of interaction between pulsed fields and cells remains elusive. We decided to examine the TLCCD profiles of cells exposed to a pulsed waveform (12).

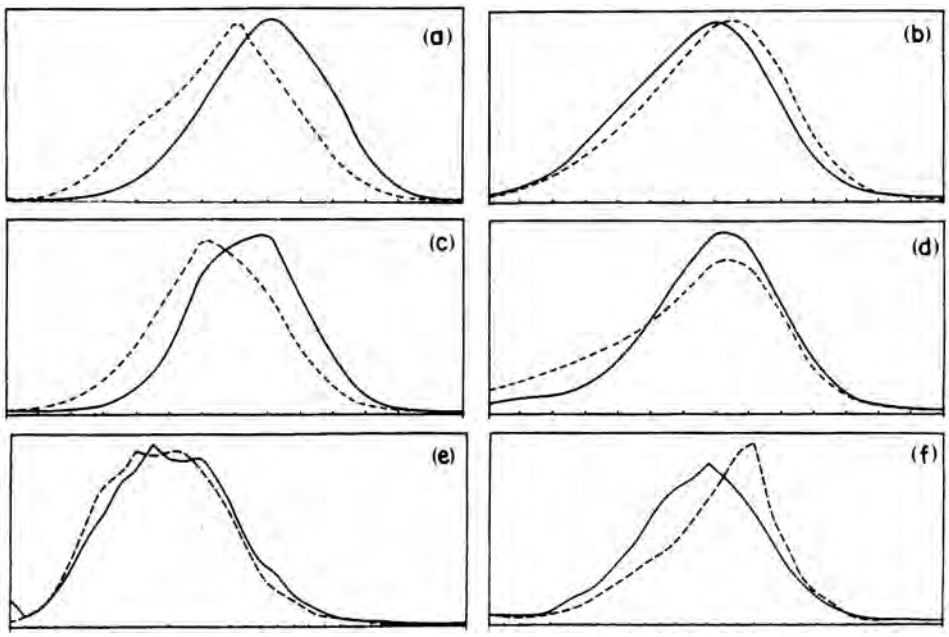

Figure 16. TLCCD profile of *Physarum* amoebae exposed for 24 hours to 60-Hz fields. The distributions occurred in a phase system in which the upper phase was positively charged relative to the bottom phase. (a) 1 V/m and 100 μT. (c) 1 V/m. (e) 100 μT. (b), (d), (f), respectively are distributions in an uncharged phase system. Dashed line, control; solid line, EMF-exposed.

Physarum amoebae were again used as the test organism, and the pulsed fields were applied using a generator and coils (Electrobiology, Fairfield, NJ). Erlenmeyer flasks containing amoebae were placed on a reciprocal shaker containing two coils. One coil was attached to the generator and the other was attached to a dummy load and served as the control. Fields were applied continuously for 24 hours as a burst of 22 sawtooth pulses of approximately 2 mT amplitude. Each pulse had a 200 sec rise and a 20 sec fall time and was separated by 5 sec; the bursts were repeated at a rate of 25 Hz. The charged and uncharged phase systems described above were used for the TLCCD experiment. The data were in agreement with our findings for cells simultaneously exposed to sinusoidal E+B fields (Figures 17a and 17b), that is, the exposed cells move to the right in the charged system and to the left in the uncharged system.

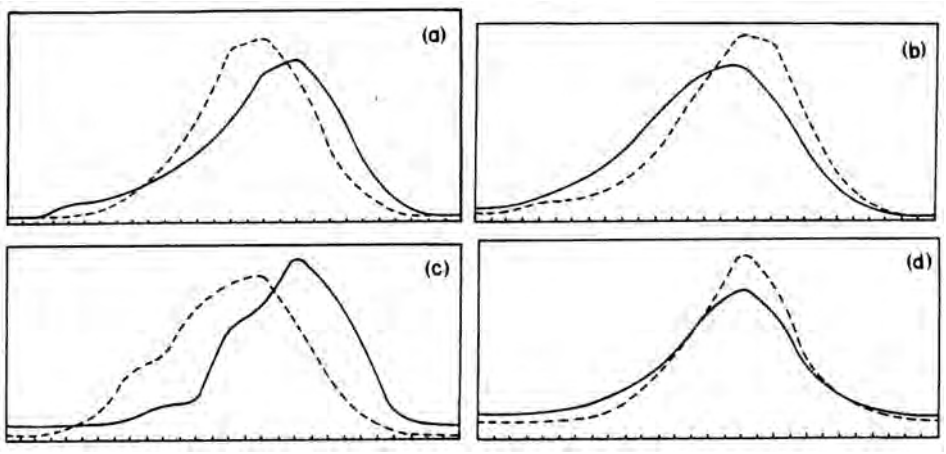

Figure 17. TLCCD profile of *Physarum* amoebae exposed for 24 hours to a pulsed electromagnetic field. Normalized cell count is plotted against the plate number. (a) and (c), cell distribution in a phase system in which the upper phase is positively charged relative to the bottom phase; (b) and (d), cell distribution in an uncharged phase system. Dashed line, control; solid line, pulse EMF-exposed.

In an experiment analogous to the sinusoidal E-only experiment described above, amoebae were exposed to a pulsed E field resembling the electric field induced by the coil system. Cells exposed to this field regimen for 24 hours were harvested and their TLCCD profiles determined. The data (Figures 17c and 17d) showed that the cells moved to the right in the charged system, but were coincident with the controls in the uncharged system. These data are consistent with the sinusoidal experiments and lend further support to the suggestion that E and B fields affect the membrane in distinctly different ways.

SUMMARY

The experiments discussed in this review admit the following conclusions:

1) Exposure of *Physarum* to sinusoidal fields of 45, 60, and 75 Hz, 0.35–1.0 V/m, and 1–200 μT lengthens the cell cycle, depresses the cell's rate of respiration and ATP content, and alters the cell membrane.

2) The time required to observe EMF effects varies from 24 hours to greater than 120 days depending on the particular parameter being examined and the mo de of fields being applied.

3) The individual fields (E field and B field) appear to be interacting by different mechanisms.

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